

Agilent Bioanalyzer
Nano Chip Protocol

Materials

- Nuclease Free water
- RNA 6000 ladder (Ambion cat. #7152)
- RNaseZAP for electrode decontamination (Ambion cat. # 9780)
- Microcentrifuge tubes (RNase free): 1.5 ml
- MJ research 0.6ml tubes for incubation
- Vortex mixer
- Chip Priming Station
- RNA 6000 Nano kit (Cat# 5065-4476): includes RNA Dye Concentrate (Blue), RNA 6000 Nano Marker (Green), RNA Gel Matrix (Red), Spin Filters, 25 RNA Nano chips per box, 2 Electrode cleaners, syringe kit.

Essential Practices:

- When dispensing into the chip wells the pipette tip must be inserted to the bottom of the well. Placing the pipette at the edge of the well leads to bubbles and poor results.
- Remove bubbles by gently tapping the chip on the bench top.
- Keep all reagents and reagent mixes refrigerated at 4°C when not in use.
- Allow all reagents to equilibrate to room temperature (10-15 minutes) before use.
- Protect dye and dye mixtures from light. Remove light covers only when pipetting. Dye decomposes when exposed to light.
- Prepared (gel-loaded) chips must be used within 5 minutes to avoid evaporation and poor results.
- Use a new syringe with each new kit.
- Use a new cleaning chip with each new kit.
- The RNA ladder must be heat denatured before each use. Aliquot 1 µl ladder into a RNase-free microcentrifuge tube and heat at 70°C for two minutes. Store on ice.

Reagent Preparation:RNA Gel Matrix (Red)

- Make fresh Gel Dye just before use, use within 2 days max.
- Pipet 550 µl of room temperature RNA Gel Matrix (Red), after vortexing, into top receptacle of spin filter.
- Centrifuge at 1500xg (4000 rpm) for 10 minutes.
- Discard spin filter, date and label this tube “Gel Matrix”
- Do not use after one month.

Gel-Dye Mix

- Put 65 µl filtered Gel Matrix in a clean 1.5 ml microcentrifuge tube.
- Vortex RNA Dye Concentrate (Blue) for ten seconds and add 1 µl RNA Dye Concentrate (Blue) to the aliquoted Gel Matrix.
- Vortex thoroughly and inspect to ensure proper mixing

- Centrifuge for 10 minutes at 13,000xg (14,000 rpm).
- Cover with foil, label “Gel Dye” and date. Use within two days.

Decontaminating the Electrodes

- Open the lid to the Bioanalyzer.
- Pull out present electrode assembly and insert electrode assembly used only for RNA.
- *Optional ethanol wash:*
 - Slowly fill one of the wells of an electrode cleaner chip with 350 μ l 100% ethanol.
 - Open the lid and place the electrode cleaner in the chip slot.
 - The chip fits one way, do not use force, close the lid and leave it closed for 1 minute.
 - Open the lid and remove the electrode cleaner. The same cleaner chip can be reused for all the chips in the kit.
- *Necessary decontaminating steps:*
 - Slowly fill one of the wells of an electrode cleaner chip with 350 μ l RNaseZAP.
 - Open the lid and place the electrode cleaner in the chip slot.
 - Close the lid and leave it closed for 1 minute.
 - Open the lid and remove the electrode cleaner.
 - Slowly fill one of the wells of another electrode cleaner with 350 μ l RNase-free water.
 - Place electrode cleaner in the Agilent 2100 Bioanalyzer.
 - Close the lid and leave it closed for 10 seconds.
 - Open the lid and remove the electrode cleaner chip.
 - Wait another 10 seconds for the water on the electrodes to evaporate.
 - Remove the cleaning solutions from the cleaner chips at the end of the day.
 - It is recommended that the RNase-free water be replaced between chips.

Sample Preparation

- Thaw RNA samples on ice.
- Dilute samples to 100-400 ng/ μ l.
- Dispense 1 μ l of RNA into each PCR tube, each chip allows analysis of 12 samples. Label tubes appropriately.
- Aliquot Ladder as indicated above.
- Heat denature samples at 70°C for at least 2 minutes. Put on ice 2 minutes.
- Spin briefly to collect sample at the bottom of the tube.

Loading the Gel-Dye Mix

- Take a new chip out of its sealed bag.
- Place the chip on the Chip Priming Station. Make sure the station base plate is in position C before loading (should always be in this position). Make sure the adjustable clip is in the upper position and the syringe is pulled up to the 1.0 mark.
- Pipette 9 μ l of Gel Dye Mix, place the tip of the pipette at the bottom of the well marked G (dark letter) and dispense.

- Close the Chip Priming Station (must hear a click) and press the plunger until it is held by the syringe clip.
- Wait exactly 30 seconds and then release the plunger with the clip release mechanism, the plunger should quickly move upward (if not, the syringe is probably cracked and needs to be replaced). Pull the plunger back to the 1 ml position.
- Open the Chip Priming Station.
- Turn over the chip to check for air bubbles. They will be obvious. Otherwise the back of the chip should look dark and homogeneous.
- Pipette 9.0 μ l of the gel-dye mix in each of the wells marked G (light letter).

Loading a Primed Chip

- Pipette 5 μ l RNA 6000 Nano Marker (Green) into each well, including the well marked with a ladder.
- Pipette 1 μ l denatured RNA sample into each well, one sample per well.
- Pipette 1 μ l denatured ladder into the well marked with a ladder
- Each well should have a total of 6 μ l. (except wells marked with a G)
- If there are unused wells, they must be filled with a total of 6 μ l RNA 6000 Nano Marker (Green) without sample.
- Place the chip gently into the chip vortexer. Lead with the bottom edge and push the top of the chip all the way into the foam chip-shaped receptacle.
- Be sure the top of the chip is flush with the top of the vortexer, or it may work its way out during vortexing.
- Vortex at the Set Point indicated on the right side of the dial for one minute.
- Carefully wipe up any spilled reagent with a KimWipe.

Running the Chip

- Open the lid to the Bioanalyzer and double-click on the “Agilent 2100 Biosizing” software.
- Place the chip into the receptacle, the chip fits one way. Don’t use force. Carefully close the lid.
- The software screen will show a picture of a chip if it is recognized.
- Select the appropriate assay from the Assay menu (mainly for our purposes the Eukaryote Total RNA Nano 2003 is the appropriate assay). Note: if there are two programs identical except for a date, use the dated or most current program.
- Click on the START icon.
- The START dialogue box appears. The type of assay will be designated in the window. The file profile will say: BioSizing. Put the cursor in front of the Biosizing prefix and type your chip number and your initials (ex. chip58DD). The extension to this file name automatically includes the date and time.
- Click the START button to begin the assay.
- Complete the sample name table when it pops up and press OK.
- If the error message “Voltages out of range” occurs, there is not enough liquid in the wells. Prepare another chip.
- After the run begins, the start button on the screen changes to a STOP. You may click on it if you need to end the run for any reason.

- To view results for individual wells as data is acquired or after the run is finished, click a well in the chip, a single well displayed on the large 12-well display, or a lane in the gel. Data regarding that well appears in a result table at the bottom of the display.
- When the assay is complete, remove the chip from the receptacle of the Bioanalyzer and dispose. Clean with RNaseZap and H₂O as before, ethanol cleaning optional.
- After cleaning, remove the RNA electrode and replace with the other electrode assembly.
- Print results for all wells, single image, gel-image, and individual electropherograms if desired. Fill out log book and store results in provided binder.

Troubleshooting:

- *If the Bioanalyzer does not recognize the chip or the chip reader there will be a gray outline of the chip reader in the upper left corner of the screen.*
 - If this happens, select the “Tools” menu from the taskbar.
 - Pull the tools menu down and select “Options”
 - From the Options screen, click on the tab that says “Reader”
 - Be sure the port selected is “COM 2”
 - If another port is selected, the software will not recognize the chip reader or the chip.
- *If partway during the run, an error window appears that says “Poor Chip Performance.” You will have to use another chip. It is possible to salvage your samples and ladder if you act quickly.*
 - Remove the chip from the chip reader and prime another chip.
 - Add 9 ul Gel Dye to the wells marked “G”
 - With a pipette set at 6ul carefully transfer all sample from each well to the new chip, being careful not to introduce bubbles.
 - Take note if the sample transferred does not equal 6 ul, and add 1 ul RNA 6000 Nano Marker (Green) if needed.
- *If there is an error message on the ladder saying that some peaks are missing, it may be possible to alter the settings to include all peaks necessary.*
 - Click on the lane of the gel-like image representing the ladder.
 - In the bottom right corner, click on the tab labeled “Settings”
 - Lower the Minimum Peak Height to include more ladder peaks.
 - Click “Apply” to apply these changes. The error flag should disappear and the samples should have peak ratios.

Uploading Bioanalyzer Data:

- Open the Agilent Bioanalyzer program
- Open the file of the chip you want to upload.
- Select “File → Print → Gel-like only.”
- Select “PDF Writer” as printer
- Name Gel-like image as “Chip #”
- Save in “D: Bioanalyzer PDF files: *Chip #*”
- Select first electropherogram for uploading.

- Select “Print → electropherogram.” Deselect any other print options, i.e. “Gel Like Image”. Choose “selected well” and print to the PDF writer.
- Name appropriately (i.e. sample name) and save in “D: Bioanalyzer PDF files” in the folder created for that chip #.
- Do this for each electropherogram.
- When the files are saved, go to MAPS online. <https://dir-apps.niehs.nih.gov/maps/>
- Select “Administer MAPS.”
- Select “Add Bioanalyzer Chip.” Fill in “Date Ran” and “Ran by” fields.
- Click on “Browse” and select the “Gel-like image” you have saved previously, it should have been named “Chip #” and saved in D: Bioanalyzer PDF files: Chip#.
- Name this file with its Chip #
- Click on “Upload”
- Return to the main menu.
- Select “Enter/Update Experiment Info”
- Select “Load Bioanalyzer PDF files”
- Browse D:/Bioanalyzer PDF files → folder named “Chip#” and select the appropriate electropherogram.
- Select the Chip # and user from the pull down list.
- Select lane # from the pull down list
- Select the appropriate RNA from the pull down list.
- Click on “Upload”
- Use the back button to return to the uploading page.
- When finished, exit Internet Explorer.